



Nanopore Analysis

USER GUIDE

14th July, 2014

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1. Introduction

Nanopore Analysis is free software built for people who struggles in data analysis of nanopore study. It provides an automatic and accurate process for measuring the dwell time and current amplitude of every individual blockades. With the help of this program, management of data, detection of events and statistical analysis could be achieved easily and conveniently. Following this guide, you will certainly have a clear idea on how to analyze your nanopore data through this software.

Methods based on different strategies are applied to recover measure or evaluate the dwell time and amplitude of events. Considering the difference of criterions or purposes for data analysis, all of the methods in this software are optional. In addition, three user parameters offered by the software, which can be used to adjust the sensitivity of blockades locations and accuracy of measurements.

If you find this software cannot meet your needs in data analysis or encounter an error in usage, please feel free to inform us by E-mail (yitaolong@gmail.com). The more you can tell us about the circumstances leading up to the bug, the better for us to improve the software.

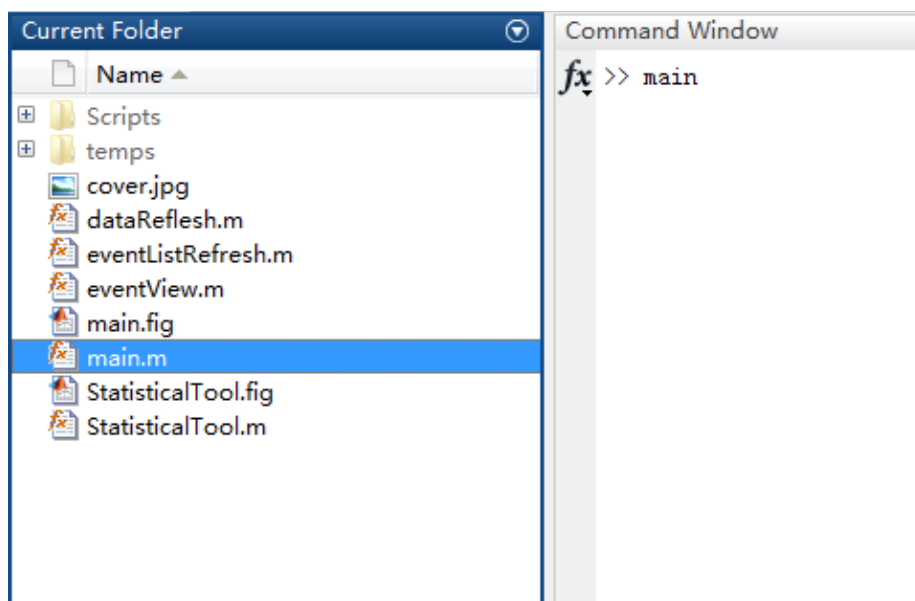
2. Get start to *Nanopore Analysis*

A [Matlab platform](#) is necessary to open this software. To ensure the software runs properly, we strongly recommended you to using a version higher than *Matlab R2008a*. So far the software can only run in Windows OS, but the next version will come with compatibility for Mac OS.

The software was packaged before uploaded. After downloading from our website, *Nanopore Analysis* should be extracted by specific tools such as Winrar, WinZip. If you do not have one, please [Click here](#) to get one. After extraction, please do not delete any files keep all the files in a same folder.

Then you can open the software in *Matlab* by either of following two ways:

- (1) Open *Matlab* -> set “Current Folder” to the folder of the software -> type in “main” in the “Command Window” -> press “Enter”;



- (2) Double click “main.m” in the folder -> the *Matlab* platform will automatically

launch -> click this icon  in the script editor of “main.m”.

3. GUI of Nanopore Analysis

Two GUIs exist in the current version, including a main GUI for data process (figure 1) and a “StatisticalTool” GUI for statistical analysis (figure 2).

The main GUI is divided into four parts. Upper half of the interface is a window for data preview. When an event is depicted in the axis, you can tune the Y-scale and X-scale to adjust the limit of the axis. In the “Data List” panel, you can load, rename and remove your data, or select a data for further analysis. In “Analysis” panel, method options and user parameters is available. The “Results” panel lists all the detected events. you can choose one to see its detailed information.

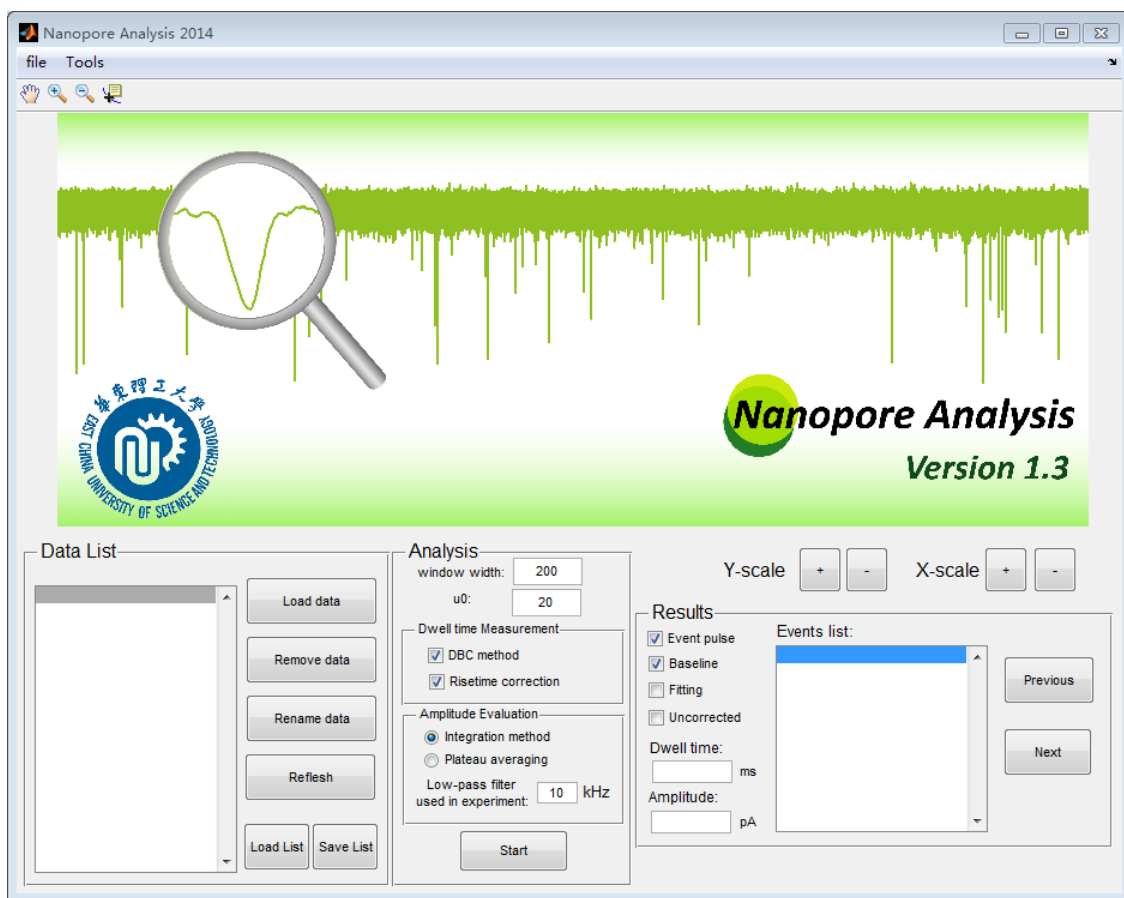


Figure 1 main GUI for data process

After detection of events, you can use the “StatisticalTool” GUI, to distribute the dwell time or amplitude of events. Data in the “selected data” list could be chosen to make a scatter or histogram.

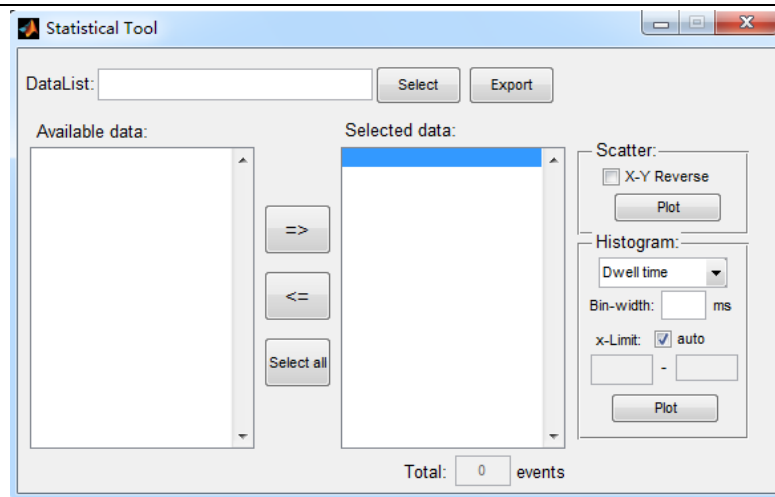


Figure 2 GUI for statistical analysis

4. Manage your data

Firstly, click the button “Load data” main GUI to select the file of your data, and then it will be imported into the data list. Note that the data file should be format of ‘.txt’ with one column of time and another column of current. The unit of time is “ms” and the unit of current is “pA”. A sample data file is available on our website ([Click here](#) to download). Similarly, you can also remove a selected data by click the button “Remove data”.

To rename your data, click the button “Rename data”, and type in a new name on the pop-up dialog. Do not to use a name which already exists in the data list.

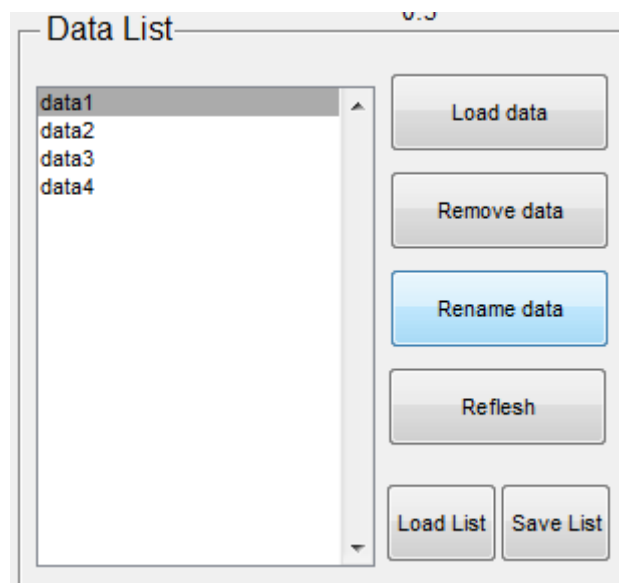


Figure 3 the “Data List” panel in the main GUI

All of the data in the list and their events information are stored in a temporary folder in the same directory of the software. If your computer or Matlab was crashed, you can try to restart the software and click the button “Refresh” to recover your data. Note that

if the software was closed in a proper way, the temporary folder will be deleted, so it is strongly recommended to save the data list after every analysis. You can just click the button “Save List”, and then a dialog will appear to ask you type in the name of list. If the name is valid, a folder will be created in the path of the software. Data as well as the detected events are saved as an individual file in the folder. Note that you can shift the directory of the folder, but not to delete files in it, especially the file “info.mat”.

Saved data list could be loaded into the software by click the button “Load list”, and selecting the corresponding folder. Be aware that the loading process will delete the unsaved data list.

5. Routine for data analysis

The first step in the data process is detection of events. A moving window method is used to evaluate baseline of the current. Default window width is 200 data points, while the user could adjust it according the noise level of the signal. The parameter u_0 is used to tune the sensitivity of detection and its default value is 20 pA. u_0 of 20 pA means events with amplitude lower than 20 pA from the baseline will not be detected.

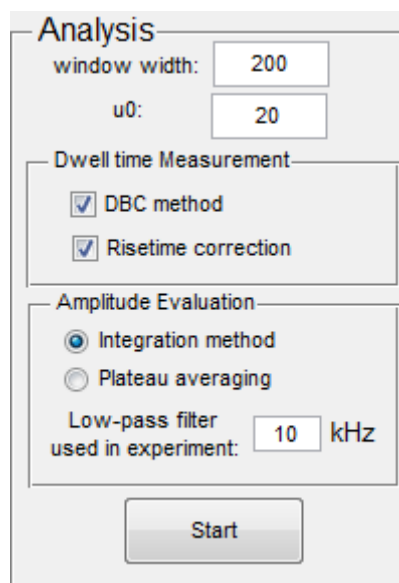


Figure 4 the “Analysis” panel in the main GUI

To achieve accurate and robust analysis, a 2nd-order differential based calibration (DBC) method was developed by our group. This method helps to correct the start and end point of the events. Risetime correction is based on an investigation which reveals the influence of filter to the shape of events. Through this method the dwell time will be modified by reset the end point of event, see reference 1 and 2. Unselecting the check box could be used to turn off the methods in data analysis.

The integration method is also developed by our group, which can evaluate the current amplitude of events. Note that the integration method should be used together with DBC method and risetime correction to ensure the result is accurate. The cutoff frequency of filter should be given correctly. Alternatively, you can also choose the plateau averaging method to measure the current amplitude.

Click the button “Start”, the software will automatically analyze the data selected in the data list, and a message box will show after finishing the analysis.

6. Preview the events

After data analysis, all the detected events are listed in the “Results” panel (figure 5). You can select one of the events, and then it will be figured in the preview window. As shown in figure 6, the corrected event pulse is depicted as red solid line; the baseline is shown in black; fitting result of event is a green dashed line and the uncorrected event is red dashed line. (reference 1) All of the elements are optional through the check box in the left of panel. In the list box, the length of event (number of data points) is demonstrated.

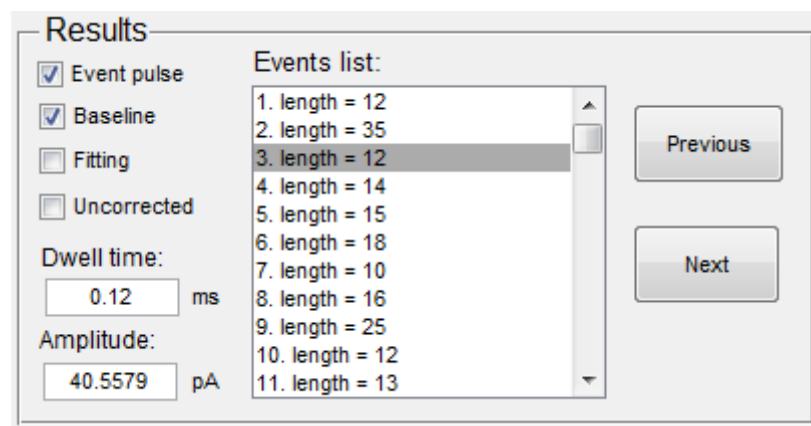


Figure 5 the “Results” panel in the main GUI

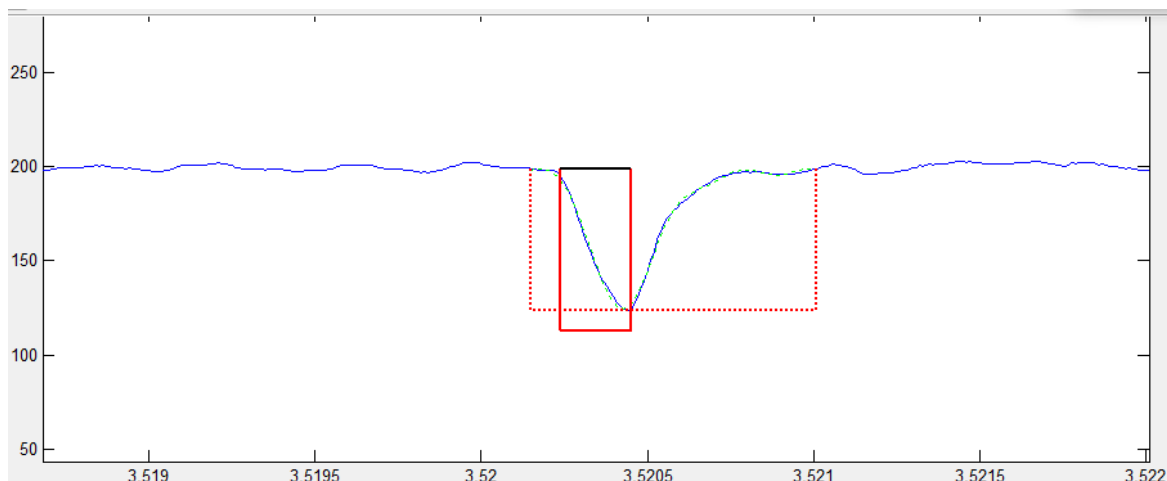


Figure 6 event depicted in the axis

7. Statistical Tool box

The statistical tool is a standalone program which can be run without the main GUI. You can open it in menu of “Tools” in the main GUI, or just type in “StatisticalTool” in the command window, and press “Enter” when the current folder of *Matlab* is set to the path of the software.

By using this tool, you should first load a data list by click the button “Select”. Data in the selected folder will be listed in the left list box. You can use the “=>”, “<=” and “select all” button to select or unselect data. The number of events is labeled on the left of the name of data linked by a hyphen. The total number of events in selected data is calculated and shown under the right listbox.

The information of dwell time and amplitude of events in selected data can be export to a excel file for analysis in other software by click the button “Export”. The left column in the excel file is the current amplitude (pA), and the right column is dwell time (ms).

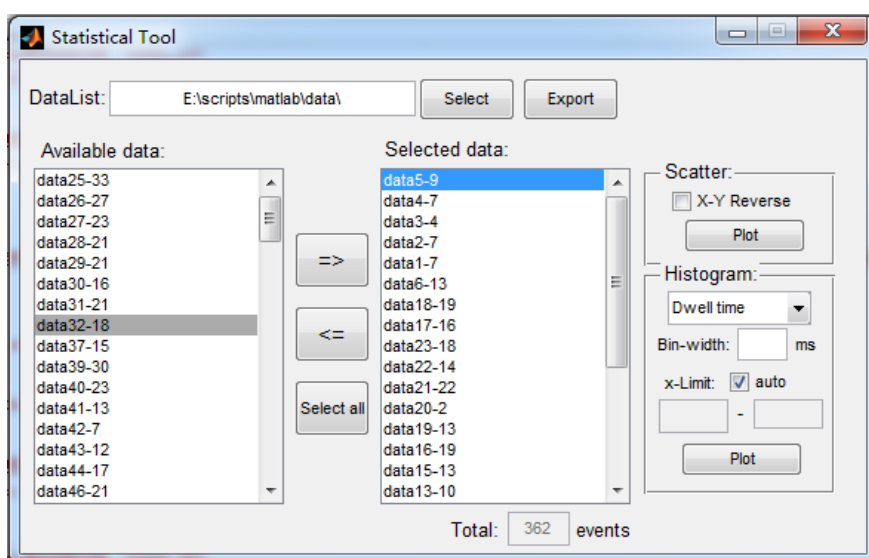


Figure 7 GUI of the StatisticalTool

Dwell time and amplitude of events in the selected data can be used to make a scatter diagram by click the “Plot” button in the “Scatter” panel. If the checkbox “X-Y Reverse” is unselected, x-axis will be the amplitude and vice versa.

The pop-up menu in the “Histogram” panel is used to select which will be distributed by histogram. Then you can set the bin-width of histogram. If the checkbox before “auto” is selected, the x-limit will be set automatically, and otherwise you can customize the x-limit in the textboxes.

8. Reference

- [1] A Data Process for Accurate and Automatic Analysis of Blockades in Nanopore Study, unpublished.
- [2] Pedone, D.; Firnkes, M.; Rant, U. *Anal Chem* **2009**, *81*, 9689.